

## CLAIMS

1. A method for transferring a nucleic acid into a cell, comprising the steps of:
  - 5 a) holding the cell under a pressure different from an atmospheric pressure; and
  - b) placing the cell and the nucleic acid under conditions capable of inducing electroporation.
- 10 2. A method according to claim 1, wherein the step of holding the cell under the pressure different from the atmospheric pressure is a step of subjecting the cell to depressurization.
- 15 3. A method according to claim 1, wherein the step of holding the cell under the pressure different from the atmospheric pressure is a step of subjecting the cell to pressurization.
- 20 4. A method according to claim 1, wherein the step of holding the cell under the pressure different from the atmospheric pressure is performed before the step of placing the cell and the nucleic acid under the conditions capable of inducing electroporation.
- 25 5. A method according to claim 2, wherein the depressurization step is performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure.
- 30 6. A method according to claim 1, wherein step b) comprises applying a high voltage pulse to the cell and the nucleic acid in at least two directions.
7. A method according to claim 1, wherein the cell is a plant cell.

8. A method according to claim 7, wherein the plant cell is a cell of dormant plant tissue.
- 5 9. A method according to claim 8, wherein the dormant plant tissue is a seed.
10. A method according to claim 7, wherein the plant is a monocotyledonous plant.
- 10 11. A method according to claim 10, wherein the monocotyledonous plant is a plant of the family *Gramineae*.
- 15 12. A method according to claim 11, wherein the plant of the family *Gramineae* is wheat (*Triticum aestivum* L.).
13. A method according to claim 11, wherein the plant of the family *Gramineae* is rice (*Oryza sativa* L.).
- 20 14. A method according to claim 11, wherein the plant of the family *Gramineae* is maize (*Zea mays* L.).
- 15 16. A method according to claim 7, wherein the plant is a dicotyledonous plant.
- 25 17. A method according to claim 15, wherein the dicotyledonous plant is a plant of the family *Cruciferae*.
- 30 18. A method according to claim 16, wherein the plant of the family *Cruciferae* is Chinese cabbage (*Brassica rapa* L.).
19. A method according to claim 16, wherein the plant of the family *Cruciferae* is rape (*Brassica napus* L.).

19. A method according to claim 15, wherein the dicotyledonous plant is a plant of the family *Leguminosae*.

5 20. A method according to claim 19, wherein the plant of the family *Leguminosae* is soybean (*Glycine max* Merr.).

21. A method according to claim 15, wherein the dicotyledonous plant is a plant of the family *Solanaceae*.

10 22. A method according to claim 21, wherein the plant of the family *Solanaceae* is tomato (*Lycopersicum esculentum* Mill.).

15 23. A method according to claim 15, wherein the dicotyledonous plant is a plant of the family *Cucurbitaceae*.

24. A method according to claim 23, wherein the plant of the family *Cucurbitaceae* is Japanese cantaloupe (*Cucumis melo* L.).

20 25. A method according to claim 15, wherein the dicotyledonous plant is a plant of the family *Convolvulaceae*.

26. A method according to claim 25, wherein the plant of the family *Convolvulaceae* is morning glory (*Pharbitis nil* Choisy).

27. A method for producing a plant, wherein a nucleic acid is transferred into cells of the plant, comprising the steps of:

30 a) holding a cell under a pressure different from an atmospheric pressure; and

b) placing the cell and the nucleic acid under conditions capable of inducing electroporation.

5 28. A method according to claim 27, further comprising a step of differentiating, growing, and/or multiplying the cell.

10 29. A method according to claim 27 or 28, wherein step a) comprises a step of holding a seed containing the cell under the pressure different from the atmospheric pressure, and step b) comprises a step of placing the seed containing the cell and the nucleic acid under the conditions capable of inducing electroporation.

15 30. A method according to claim 29, wherein the seed is a monocotyledonous plant seed.

20 31. A method according to claim 30, wherein the monocotyledonous plant seed is a seed of the family *Gramineae*.  
32. A method according to claim 31, wherein the seed of the family *Gramineae* is a wheat (*Triticum aestivum* L.) seed.

25 33. A method according to claim 31, wherein the seed of the family *Gramineae* is a rice (*Oryza sativa* L.) seed.

34. A method according to claim 31, wherein the seed of the family *Gramineae* is a maize (*Zea mays* L.) seed.  
35. A method according to claim 29, wherein the seed is a dicotyledonous plant seed.

36. A method according to claim 35, wherein the

dicotyledonous plant seed is a seed of the family *Cruciferae*.

37. A method according to claim 36, wherein the seed of the family *Cruciferae* is a Chinese cabbage (*Brassica rapa* L.) seed.

38. A method according to claim 36, wherein the seed of the family *Cruciferae* is a rape (*Brassica napus* L.) seed.

10 39. A method according to claim 35, wherein the dicotyledonous plant seed is a seed of the family *Leguminosae*.

40. A method according to claim 39, wherein the seed of the family *Leguminosae* is a soybean (*Glycine max* Merr) seed.

15 41. A method according to claim 35, wherein the dicotyledonous plant seed is a seed of the family *Solanaceae*.

20 42. A method according to claim 41, wherein the seed of the family *Solanaceae* is a tomato (*Lycopersicum esculentum* Mill) seed.

25 43. A method according to claim 35, wherein the dicotyledonous plant seed is a seed of the family *Cucurbitaceae*.

44. A method according to claim 43, wherein the seed of the family *Cucurbitaceae* is a Japanese cantaloupe (*Cucumis melo* L.) seed.

30 45. A method according to claim 35, wherein the dicotyledonous plant seed is a seed of the family *Convolvulaceae*.

46. A method according to claim 45, wherein the seed of the family *Convolvulaceae* is a morning glory (*Pharbitis nil* Choisy) seed.

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47. A plant, produced by a method according to any one of claims 27 to 46.

10 48. A plant according to claim 47, which does not contain a somaclonal variation.

49. An apparatus for transferring a nucleic acid into a cell, comprising:

15 a) a section for holding the cell under a pressure different from an atmospheric pressure; and  
b) a section for electroporation.

20 50. An apparatus according to claim 49, wherein the section for holding the cell under the pressure different from the atmospheric pressure have an ability of maintaining the pressure lower than the atmospheric pressure.

25 51. An apparatus according to claim 49, wherein the cell is a plant cell.

52. An apparatus according to claim 49, wherein the electroporation section of b) comprises:  
a first electrode functioning as an anode; and  
a second electrode functioning as a cathode,  
30 wherein a distance between the first electrode and the second electrode is long enough to accommodate a plant seed.

53. An apparatus according to claim 52, wherein the distance between the first electrode and the second electrode is at least about 5 mm.

5 54. An apparatus according to claim 52, wherein the distance between the first electrode and the second electrode is longer than about 1 cm.

10 55. An apparatus according to claim 52, wherein the first electrode and the second electrode are platinum electrodes.

15 56. An apparatus according to claim 49, wherein the electroporation section of b) comprises a first electrode functioning as an anode and a second electrode functioning as a cathode,  
wherein a distance between the first electrode and the second electrode can be changed so that a plant seed can be accommodated between the first and second electrodes.

20 57. An apparatus according to claim 56, wherein the first electrode and the second electrode are platinum electrodes.

25 58. An electroporation apparatus for transferring a nucleic acid into a cell, in combination with holding the cell under a pressure different from an atmospheric pressure, the apparatus comprising:  
a first electrode functioning as an anode; and  
a second electrode functioning as a cathode,  
wherein a distance between the first electrode and the second electrode is long enough to accommodate a plant seed.

30 59. An apparatus according to claim 58, wherein the distance

between the first electrode and the second electrode is at least about 5 mm.

5 60. An apparatus according to claim 58, wherein the distance between the first electrode and the second electrode is longer than about 1 cm.

10 61. An apparatus according to claim 58, wherein the first electrode and the second electrode are platinum electrodes.

15 62. An electroporation apparatus for transferring a nucleic acid into a cell, in combination with holding the cell under a pressure different from an atmospheric pressure, the apparatus comprising:

15 a first electrode functioning as an anode; and  
a second electrode functioning as a cathode,  
wherein a distance between the first electrode and the second electrode can be changed so that a plant seed can be accommodated between the first and second electrodes.

20 63. An apparatus according to claim 62, wherein the first electrode and the second electrode are platinum electrodes.

25 64. An electroporation chamber capable of resisting a pressure different from an atmospheric pressure and having an enough size to accommodate a plant seed.

30 65. An electroporation chamber according to claim 64, wherein the diameter of a largest inscribed circle touching at least three points on an inner wall of the electroporation chamber is at least about 5 mm.

66. An electroporation chamber according to claim 64,

wherein the diameter of a largest inscribed circle touching at least three points on an inner wall of the electroporation chamber is longer than about 1 cm.

5 67. An electroporation chamber according to claim 64, wherein the chamber has a quadrangular horizontal section and inner dimensions of the chamber are about 1 cm x 2 cm x 2 cm.

10 68. An electroporation chamber according to claim 64, wherein the chamber has circular horizontal section and inner dimensions of the chamber are about 1 cm x 4 cm.

15 69. An electroporation chamber capable of resisting a pressure different from an atmospheric pressure, wherein a size of the chamber can be changed so that a plant seed can be accommodated in the chamber.

20 70. An apparatus for automatically performing electroporation, comprising:

- a) a container for placing a mixture containing a nucleic acid and cells;
- b) a section for placing the nucleic acid in container a);
- c) a section for placing the cells in container a);
- d) a container for holding the cells under a pressure different from an atmospheric pressure, the container being capable of resisting the pressure different from the atmospheric pressure;
- e) a section for placing the cells in container d);
- f) a section for maintaining the inner area of container d) at the pressure different from the atmospheric pressure;

- g) a container for applying a high voltage pulse to the mixture containing the nucleic acid and the cells;
- h) a section for placing the mixture containing the nucleic acid and the cells in container g);
- 5 i) a section for applying a high voltage pulse to the mixture containing the nucleic acid and the cells in container g); and
- j) a section for automatically performing section b), c), e), f), h), and i),

10 wherein section b) and section c) are the same or different from each other; section e) and section h) are the same or different from each other; and container a), container d), and container g) are the same or different from one another.